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ORIGINAL PAPER

Effect of mixed-cropping and water-stress on macro-nutrients and biochemical constituents of rhizomatous medicinal plants in Central Himalaya, India

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Abstract: Plants in the alpine zone mainly depend on the reserved food materials stored in their rhizomes for the next growing season. We investigated the effect of mixed cropping (*Phaseolus vulgaris* L. var. Pinto) with four rhizomatous medicinal plants, i.e., *Angelica glauca*, *Arnebia benthamii*, *Rheum emodi* and *Pleurospermum angelicoides* as well as three levels of water stress treatment under two conditions (shade net and open field) on macronutrients (NPK) and biochemicals (carbohydrates and protein). The experiment was conducted by completely randomized design (CDR). The data were analyzed with ANOVA as well as CDR. The experimental results show that in all the species shade conditions with sever water stress (SSWS) increased the level of macronutrients (NPK). However, (N) concentration was highest under shade with mixed cropping (SMIX). Under SMIX, carbohydrate content was highest than open field control conditions (CONT). This investigation results demonstrate that mixed cropping of medicinal plants with *Phaseolus vulgaris* could be a good livelihood option in the mountainous regions of Indian Central Himalaya. And the water-stress conditions along with mixed cropping could improve the biochemical constituents in the rhizome of these species.

Keywords: Mixed cropping; Medicinal and Aromatic plants; Nitrogen fixation; *Phaseolus vulgaris*; Water stress

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Introduction

In Central Himalaya, there is a large number of valuable medicinal and aromatic plants (MAPs) growing naturally in alpine and sub-alpine zones. These zones are characterized by cold climatic conditions and are mostly covered with snow for longer periods during the year. Due to prolonged snow cover, the growing season for plants is very short and this makes it difficult for plants to survive on the limited stored nutrient reserves (Kanai and Masuzawa 1993). Plants in these climatic zones, therefore, developed large reserve organs and utilize these nutrient reserve substances for their growth (Midorikawa 1959; Mooney and Billings 1960). These reserves serve two important functions in plants. Firstly, the stored resources offer plants with a competitive advantage to start early spring-growth before their neighbours grow (Heilmeier et al. 1986). Secondly, resources may be stored to bridge the temporal gaps between resource availability and resource demand during the growing seasons (Bloom et al. 1985; Chapin et al. 1990). The MAPs growing in alpine, sub-alpine and temperate regions provide sustainable livelihoods option for people living in high altitude region between 2,200–2,300 m asl (Olsen and Larsen 2003). However, during recent years, due to over-and illegal exploitation of MAPs, especially those in high demand, the original habitats fragmented into isolated patches leading to loss of species population in these areas (Tandon 1998). Due to this anthropogenic pressure and disturbance, it is essential to develop conservation and management strategies of these plant species to minimize exploitation from their natural habitats.

Higher altitude region of the Central Himalayas is also known for most successful mixed cropping systems, where a high percentage of agricultural land is still under mixed cropping (Maikhuri 1996). In Central Himalaya, MAPs cultivation becomes an emerging strategy in recent years to increase income of marginal farmers and as a substitute for low yielding traditional and cash crops (Chauhan et al. 2010). Mixed cropping of MAPs with *Phaseolus vulgaris* L. may be a beneficial practice in this region

(Maikhuri et al. 2000). Being leguminous crops, *P. vulgaris* play an important role in maintaining and improving the soil fertility status by incorporating atmospheric nitrogen through symbiotic association with mycobacteria through the root nodules. On the other hand, the root systems of some MAPs contribute to soil stabilization and prevent erosion in the fragile hilly slopes (Karki et al. 2003). The agriculture in high altitude of the Central Himalayan region is mostly practiced on rainfed conditions, and water availability is, therefore, an important factor in the cultivation of crop plants. Water serves a number of functions in plant growth and survival, and its availability and quantities in plant growth is very critical (Li et al. 2007). Medicinal plant cultivation under different water stress is an important factor in controlling phytochemical levels in plants (Zheng et al. 2007). However, water stress may directly limit vegetative growth during dry seasons (Reich and Borchert 1984) and water availability may alter nutrient levels such as nitrogen (N) and phosphorus (P) in a plant (Birch 1964; Tissue and Wright 1995). Though several studies were conducted on alpine and sub-MAPs growing alpine regions of the Central Himalaya on plant growth and phenology (Farooqi and Vasundhara 1997; Singh and Sundriyal 2005; Butola and Badola 2007; Vashistha et al 2009), underground growth in many such plants still received very little attention.

Considering the above-mentioned factors, our study attempted to evaluate the effects of mixed cropping with *Phaseolus vulgaris* L. var. *Pinto* and water stress on macro nutrient (NPK) and biochemical molecules of underground rhizomes of medicinally important plants: *Angelica glauca* Edgew., *Arnebia benthamii* Wall. Ex G. Don, *Rheum emodi* Wall. Ex Meissn. and *Pleurospermum angelicoides* DC. C.B. Clarke. The experiments were conducted under various condition, *viz.* control, shade net and open field conditions.

Materials and methods

The study area

The study was conducted in the Suraithota village (2,180 m asl) stretch of the Dhauli Ganga catchment of Nanda Devi Biosphere Reserve (NDBR) (30°17' N and 30°41' N and 79°40' E and 80°5' E) in the Central Himalaya, India. The area is one of the biologically most diverse regions with a repository of a large variety of MAPs (Fig.1). Monthly average (minimum and maximum) temperature and rainfall of two years under open field and shade net conditions were recorded over a two-year period (2002 to 2003) (Fig. 2).

Growth conditions

Mixed cropping

Phaseolus vulgaris was grown as a mixed crop with each of the species namely *A. glauca*, *A benthamii*, *R. emodi* and *P. angelicoides* under shade net and open field condition (OMIX).

Open condition

Open field space without any cover or modifications was taken as control. Plants grown without mixed-cropping under open field condition were treated as control (CONT).

Fig. 1 Map of the study site (Not to the scale)

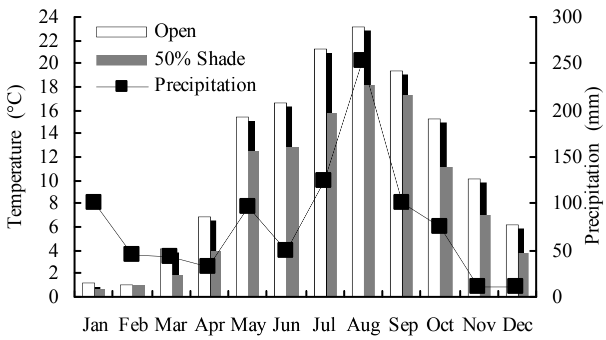

Fig. 2 Temperatures and rainfall under open field and shade condition.

Shade conditions

A shade net made of sheets of 50% Nelton agronets (Parry and Co. Ltd Vadodara, Gujarat, India) were erected on a bamboo frame that was 10 m length, 2 m width and 3 m height. The top and the sides were covered with 50% shading net (green; Nelton agronets). Our experiment included treatments with mixed cropping and different water stress (control, moderate and severe). SMWS is short for plants grown under moderate water stress, SSWS for severe water stress, and SMIX for under shade with mix cropping.

Water stress treatments

The experiment included three treatments with different water

stress levels: control (CONT) with irrigation every day, Open with moderate water stress (OMWS) with irrigation twice weekly, Open with sever water stress (OSWS) with irrigation once weekly. The design of experiment was done using Completely Randomised Design (CDR). The data were analyzed using ANOVA as well as CDR.

Plant material

Sixty plantlets of each *A. glauca*, *R. emodi*, *P. angelicoides* and *A. benthamii* were raised through vegetative cuttings of the same age. These cuttings were grown in open field conditions for twelve months without any fertilizer treatments and then transplanted to each condition (shade net and open field), where they grew with mixed cropping and different water stress treatments for a further period of two years. Ten plants from each species were randomly selected and uprooted from each treatment at the end of the first and second harvest. All samples were washed in running tap water to remove the soil and blotted dry. The plant samples were dried in surface and the fresh weight was recorded. The rhizomatous parts of the plants were dried at 80 °C for 3 days. The dried plant material were ground to fine powder and sieved. The fine powder was then used for macro nutrient and biochemical estimation.

Estimation of macronutrients (NPK)

Nitrogen (N) was estimated following a modified Kjeldhal method (Allen 1989), and phosphorus (P) was estimated by colorimeter determination by molybdate reagent and ascorbic acid, and absorbance was measured at 880 nm in a UV Spectrascan unit (Anderson and Ingram 1993). Potassium (K) was measured with flame photometer.

Total Soluble Protein

Total soluble protein content was estimated using the dye binding principle method (Bradford 1976). Plant material (50 mg) of rhizomatous was homogenized with 10 mL extraction buffer (0.1 M, Tris-HCl pH 7.5, 2 mM ethylene diamine tetra acetic acid (EDTA)) and 0.1% β-mercaptoethanol) at 4°C. During homogenization, a pinch of phenyl methane sulphonyl fluoride (PMSF) was added to prevent proteolysis. All the homogenates were centrifuged at 10,000 rpm for 30 min at 4°C. The clear supernatants were collected and used for protein analysis. The supernatant was mixed with 4.9 mL of Bradford reagent (0.01% w/v Coomassic Brilliant Blue G-250, 5% (v/v) ethanol and 10% (v/v) orthophosphoric acid). After cyclomixing, the reaction mixture was incubated at room temperature for 30 min and then cyclomixed again. The absorbance of the reaction mixture was measured at 595 nm against a reagent blank. A standard curve was obtained by using bovine serum albumin (Sigma) as a reference.

Carbohydrate estimation

The amount of total soluble sugar and starch were estimated using the McCready method (McCready et al.1950).

Soluble sugar

The amount of total soluble sugar was estimated using the McCready method, (McCready et al. 1950). Dried powder (50 mg) of plant material was homogenized with 5 mL hot 80% ethanol at 80°C. Samples were centrifuged at 3,000 rpm for 15 min, supernatant separated, decolorized with activated carbon and filtered. The supernatants were treated using the anthrone method (0.2% anthrone in cold 95% H_2SO_4) and heated for 7 min in boiling water bath. The absorbance of the sample after cooling was measured at 620 nm (OD 620) in a Beckman DU-65 Spectrophotometer (Beckman Instruments, Palo Alto, CA). Total sugar concentrations of the samples were calculated using the calibration curve drawn for (standard) glucose solutions (Ebell 1969).

Soluble starch

The residues left after the extraction of soluble sugars was used for starch estimation. The residues were kept dry in a cold place (10°C) overnight, suspended in 5.0 mL water, and subsequently 6.5 mL 52% (w/v) perchloric acid was added and the sample centrifuged at 3,000 rpm for 20 min. The supernatant was decanted and collected. The whole procedure was repeated thrice. Absorption of the samples was read at 630 nm. Starch concentration was calculated using the calibration curve drawn for a (standard) glucose solution (Mc Cready et al. 1950).

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) using Gen Stat 12th Edition statistical package. Duncan's multiple range test at 5% level (p < 0.05) was used to separate significant differences between means of treatments.

Results

Estimation of NPK

In *R. emodi,* nutrients varied from one treatment to another. After the first and final harvest, nitrogen (N) was found highest under SMIX treatment (Table 1). On the first harvest, phosphorus (P) was found highest under OMWS after first and second harvest, respectively (Table1). Potassium (K) was highest under OSWS treatment after first harvest. However, on second harvest it was obtained higher under SSWS treatment. In *P. angelicoides*, N was highest under CONT treatment on first harvest and under OMWS treatment on second harvest (Table 2). Phosphorus was higher under OMIX and OMWS in first and second harvest, respectively (Table 2). On the other hand, K was found higher under SSWS on the first harvest and under SMIX treatment on second harvest. In *A. benthamii*, N was highest under CONT treatment on first harvest and OMWS after second harvest (Table 3). Phosphorus was exhibited highest under SMIX treatment on first and second harvest (Table 3). However, K was highest under

OSWS treatment on the first harvest and under OMIX treatment on second harvest.

On first harvest in *A. glauca*, N was higher under OMWS treatment and under OSWS on the second harvest (Table 4). Phosphorus was higher under OMWS and CONT on first and second harvest, respectively (Table 4). Potassium was recorded higher under SSWS treatment on first harvest and under SMIX on second harvest.

Table 1. NPK status of *Rheum emodi* after first and second harvest

Treatment	Nitrogen (%)		Potassium (%)		Phosphorus (%)	
	First harvest	Final harvest	First harvest	Final harvest	First harvest	Final harvest
CONT	1.95 ± 0.39 d	2.54 ± 0.02 b	0.66 ± 0.01 d	0.96 ± 0.01 c	0.176 ± 0.03a	0.222 ± 0.03e
OMWS	2.52 ± 0.09b	1.93 ± 0.64 d	0.82 ± 0.01 c	1.30 ± 0.01 a	0.633 ± 0.01a	0.782 ± 0.01 a
S MWS	2.62 ± 0.01 b	1.93 ± 0.08 d	0.45 ± 0.01 e	0.93 ± 0.01 c	0.423 ± 0.01c	0.613 ± 0.01 b
OSWS	2.83 ± 0.06 a	1.70 ± 0.01 e	1.41 ± 0.01a	1.23 ± 0.01 b	0.443 ± 0.01 c	0.461 ± 0.01 d
S SWS	2.1 3± 0.01 c	2.66 ± 0.08 c	0.84 ± 0.01 c	1.71± 0.02 a	0.531 ± 0.03b	0.451 ± 0.01 c
O MIX	2.47 ± 0.01 b	2.03 ± 0.01 cd	1.25 ± 0.02 b	0.78 ± 0.18 d	0.516 ± 0.01 b	0.534 ± 0.01 c
S MIX	2.86 ± 0.01 a	3.61 ± 0.14 a	1.26 ± 0.03 b	0.73 ± 0.01d	0.323 ± 0.01 d	0.242 ± 0.01e

Results are represented as means (± standard error). Values with the same letters do not differ at a 5% level of significance ($p \leq 0.05$).

Table 2. NPK status of *Pleurospermum angelicoides* after first and second harvest

Treatment	Nitrogen (%)		Potassium (%)		Phosphorus (%)	
	First harvest	Final harvest	First harvest	Final harvest	First harvest	Final harvest
CONT	3.79 ± 0.16 a	4.70 ± 0.03 c	1.49 ± 0.01 b	1.89 ± 0.01 d	0.503 ±0.05 bc	0.473 ± 0.01 b
OMWS	2.68 ± 0.01 c	5.21 ± 0.01 a	1.52 ± 0.02 b	2.26 ± 0.012b	0.204± 0.01 f	0.593 ± 0.01 a
S MWS	3.01± 0.05 b	1.98 ± 0.04 f	1.76 ± 0.03 a	1.86 ± 0.03 d	0.282 ± 0.01 e	0.446 ± 0.01b
OSWS	3.66 ± 0.16 a	5.02 ± 0.07 b	0.99 ± 0.04 c	1.28 ± 0.02 f	0.541 ± 0.02bc	0.452 ± 0.01 b
S SWS	2.66 ± 0.08 c	2.30 ± 0.01 d	1.72 ± 0.01 a	1.63 ± 0.03 e	0.451 ± 0.01 c	0.331 ± 001 c
O MIX	2.12 ± 0.04 e	2.12 ± 0.01 ef	1.47 ± 0.05 b	2.09 ± 0.06 c	0.716 ± 0.01 a	0.333 ± 0.01 c
S MIX	2.34 ± 0.02 d	2.17 ± 0.01 de	1.76 ± 0.06 a	2.62 ± 0.05 a	0.383± 0.01 d	0.286 ± 0.01 d

Results are represented as means (± standard error). Values with the same letters do not differ at a 5% level of significance ($p \leq 0.05$).

Table 3. NPK status of *Arnebia benthamii* after first and second harvest

Treatment	Nitrogen (%)		Potassium (%)		Phosphorus (%)	
	First harvest	Final harvest	First harvest	Final harvest	First harvest	Final harvest
CONT	1.86 ± 0.07 a	2.83 ± 0.22 a	1.08 ± 0.1 d	2.21 ± 0.01a	0.371 ± 0.01 c	0.243 ± 0.07 c
OMWS	1.72 ± 0.02 b	2.88 ± 0.03a	1.13 ± 0.01 d	1.67 ± 0.02 d	0.702 ± 0.05 b	0.226 ± 0.02 c
S MWS	1.48 ± 0.04 c	2.25 ± 0.03b	1.57 ± 0.01 b	1.48 ± 0.02 e	0.311 ± 0.02d	0.163 ± 0.01 d
OSWS	1.41 ± 0.03 c	2.75 ± 0.01 a	2.11 ± 0.01 a	1.88 ± 0.01 b	0.715 ± 0.01b	0.193 ± 0.07d
S SWS	1.72 ± 0.01 b	2.08 ± 0.02 bc	1.22 ± 0.02 c	1.46 ± 0.02 e	0.682 ± 0.08 b	0.126 ± 0.02 e
O MIX	1.65 ± 0.02 b	1.80 ± 0.03c	1.29 ± 0.01 c	2.22 ± 0.01 a	0.694 ± 0.01 b	0.546 ± 0.02 b
SMIX	1.66 ± 0.01 b	1.96 ± 0.02bc	1.64 ± 0.06 b	1.74 ± 0.01 c	0.842 ± 0.02 a	0.656 ± 0.15 a

Results are represented as means (± standard error). Values with the same letters do not differ at a 5% level of significance ($p \leq 0.05$).

Table 4. NPK status of *Angelica glauca* after first and second harvest

Treatment	Nitrogen (%)		Potassium (%)		Phosphorus (%)	
	First harvest	Final harvest	Fist harvest	Final harvest	First harvest	Final harvest
CONT	2.72 ± 0.02c	3.22 ± 0.04 d	0.57 ± 0.01d	0.96 ± 0.93 c	0.318 ± 0.01 e	0.642 ± 0.03 a
OMWS	3.70 ± 0.17 a	5.13 ± 0.01 b	1.09 ± 0.02 a	1.02 ± 0.02 c	0.782 ± 0.01 a	0.131 ± 0.06 d
S MWS	2.41 ± 0.12d	4.51± 0.02c	0.97 ± 0.03 b	0.45 ± 0.01 e	0.621 ± 0.01c	0.272 ± 0.02 e
OSWS	2.11 ± 0.05 e	5.61 ±0.01 a	0.87 ± 0.04 b	1.19 ± 0.02 a	0.733 ± 0.01 b	0.453 ± 0.08 b
S SWS	3.02 ± 0.02 b	3.32 ± 0.05d	1.11 ± 0.06 a	1.12 ± 0.03 b	0.274 ± 0.02 e	0.353 ± 0.01 c
O MIX	3.23 ± 0.01 b	2.21 ± 0.05 e	0.69 ± 0.01 c	0.66 ± 0.01 d	0.691 ± 0.08 b	0.453 ± 0.01 b
S MIX	2.96 ± 0.02 bc	1.76 ± 0.6f	0.97 ± 0.03 b	1.23 ± 0.03 a	0.462 ± 0.03 d	0.356 ± 0.01 c

Results are represented as means (± standard error). Values with the same letters do not differ at a 5% level of significance ($p \leq 0.05$).

Carbohydrate and protein estimation

In *R. emodi,* on the first harvest, protein content was higher under CONT treatment followed by SMIX. However, the sugar content was highest under SMIX followed by OMIX, and starch was highest under OSWS treatment after first harvest (Fig. 3) and exhibited significant difference at (p <0.05). After final harvest protein was found highest under SMIX, sugar was found under OMIX and starch was obtained highest under OSWS treatment (Fig. 4). In *P. angelicoides* after the first harvest, protein was highest under SMWS, and sugar was highest under OMIX treatment and starch was highest under SMIX treatment (Fig. 1). After the second harvest, protein was highest under SSWS, sugar and starch was found highest under OMIX treatment and exhibited significantly different (Fig. 4). In *A. benthamii* after the first harvest, protein was highest under OMWS treatment, and sugar and starch were highest under SMIX treatment and were significantly different (Fig. 3). However, after the second harvest, protein was highest under CONT treatment, sugar was found highest under SMWS and starch was highest under OMIX treatments (Fig. 4). In *A. glauca* after the first harvest, protein and sugar was found highest under OMIX treatment. However, starch was highest under SMIX treatment after first harvest (Fig. 3). In second harvest, protein was highest under OMIX treatment, sugar under SMIX and starch under OMWS treatment (Fig. 4).

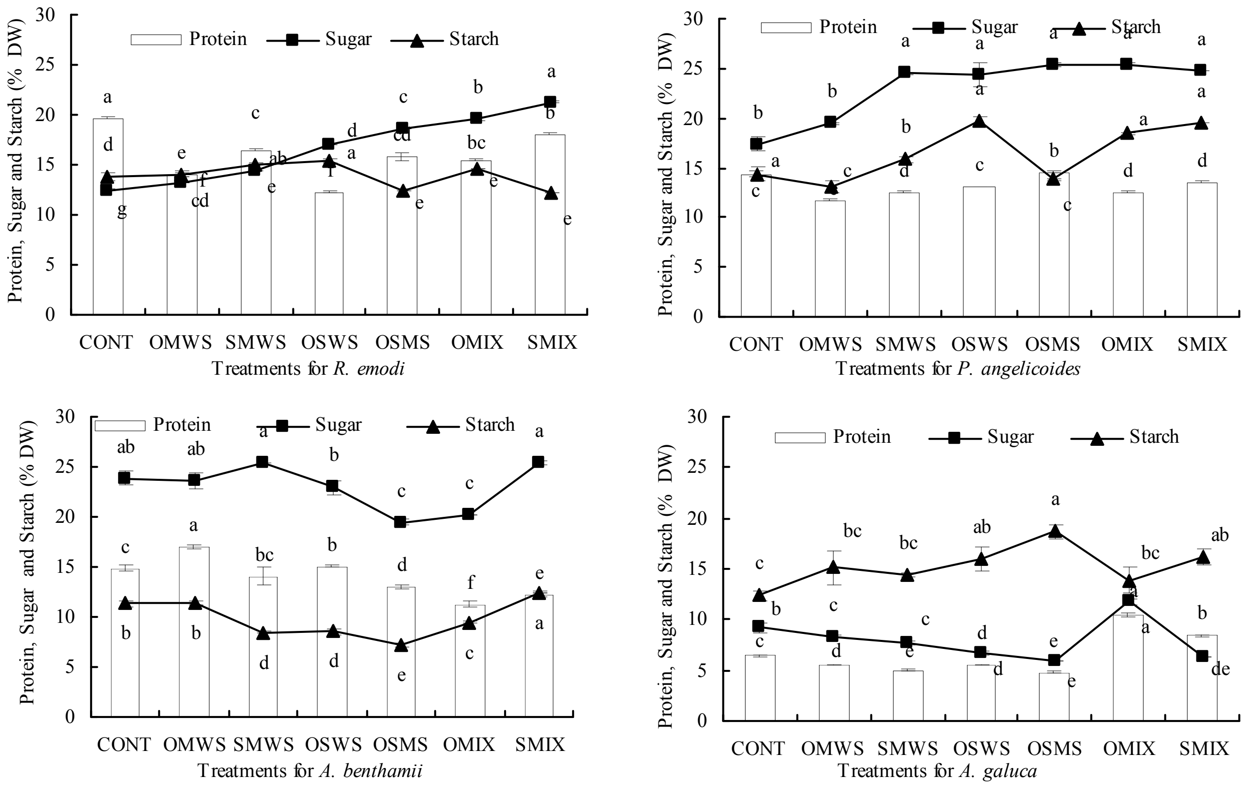

Fig. 3 Effect of water stress and mix cropping on Carbohydrates (sugar and starch) and Protein after first harvest. Bars with different letter (s) are significantly different ($p \leq 0.05$).

Discussion

The results indicate that N was higher under SMIX treatment in all the species. However, N is one of the key limiting factors in crop production, and availability of this nutrient fluctuates greatly under mixed cropping (Haynes and Francis 1990). However, the large amounts of N available in the rhizome are not utilized during the seasonal growth (Jaeger and Monson 1992). Lipson et al. (1996) reported that the luxury uptake of nutrients allow plants to capitalize on nutrient on pulses, when concurrent growth is not possible. About 60% of annual above ground N requirement is met by translocation of previously stored N from the rhizome (Jaeger and Monson 1992). However, P was highest under OSWS and SMIX treatment in all the species, as water stress may be severe in alpine ecosystem (Mooney et al. 1965; Oberbauer and Billings 1981; Jackson and Bliss 1982). Sayed et al. (2008) also reported that active constituents significantly increased under moderate water stress condition compared with control conditions. The results highlight that moderate water stress (MWS) and mixed cropping enhance the macro nutrient uptake in all the species.

There was an increase in carbohydrates (sugar and starch) content on the first harvest. As the growing season of *A. glauca*, *A. benthamii*, *R. emodi* and *Pleurospermum angelicoides* are very short (April to September), the shoots and aerial parts senesce by early October before the beginning of considered season. However, these plants may store carbohydrates reserves during the summer and rainy season for their growth and biomass yield (Kanai and Masuzawa 1993). A seasonal change in carbohydrate concentration in some plants are reported (Bonicel et al. 1987; Ashworth et al. 1993; Bhowmik and Matsui 2003). High underground reserves late in the season are due to translocation from shoots (Mooney and Billings 1960). High level of soluble carbohydrates in rhizomes may be necessary for resistance to cold and winter in the arctic (Russell 1948). After second harvest, starch level in *A. glauca*, *R. emodi* and *P. angelicoides* were relatively higher. However, the sugar was low under in all the species under SMIX treatment. This may be due to sugar conversion into starch during winter and subsequently sugar may accumulate during summer. Sugar and starch concentration were higher in rhizome but varying in distribution of the species from different location (Gallagher et al. 1984). Increase in sugar concentration in alpine acts as osmoregulation against the protection from the low temperature (Körner 1999).

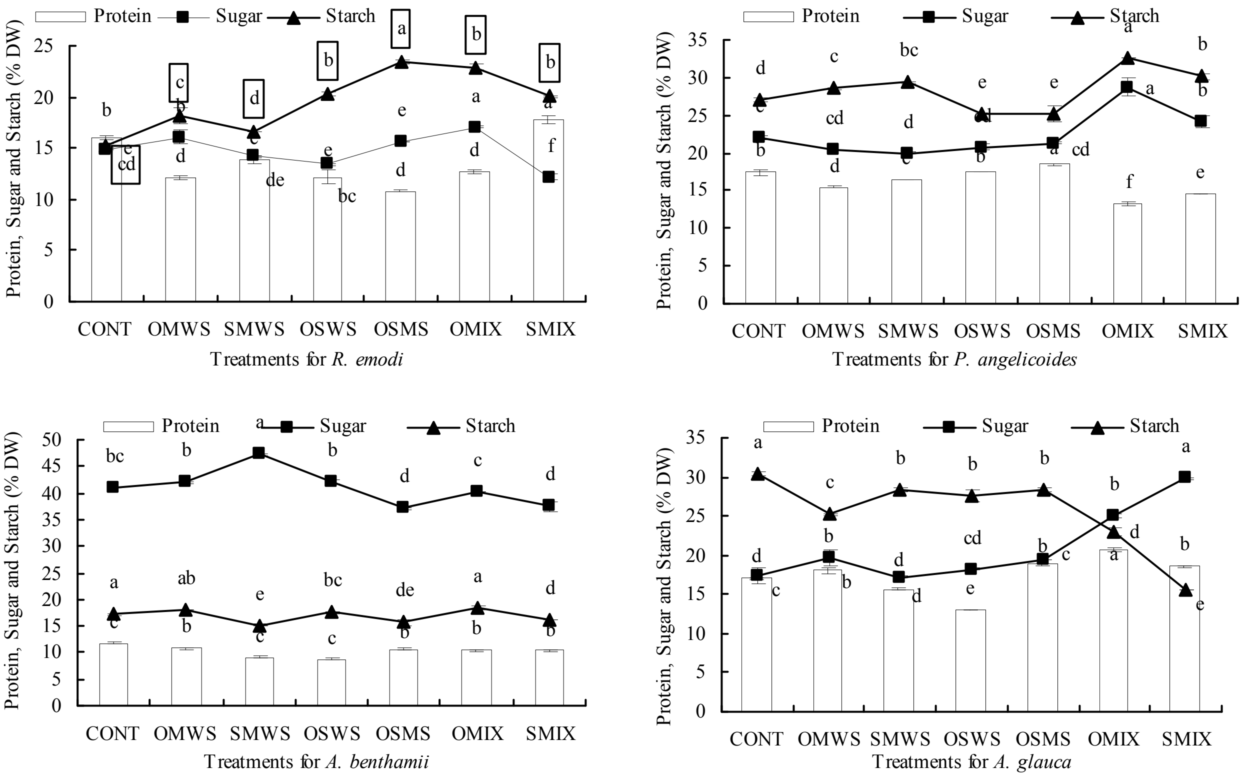

Fig. 4 Effect of water stress and mix cropping on Carbohydrates (sugar and starch) and Protein after second harvest. Bars with different letter (s) are significantly different ($p \leq 0.05$).

Conclusion

Although environmental conditions in this experiment were different from those of the natural habitats of these plant species. The results in this investigation highlight that under severe water stress and mixed cropping conditions there is an increase in the biochemical constituents of MAPs in the high altitude region, where agricultural is totally practiced on rainfed conditions. Local inhabitants and farmers of this marginal mountain area are still looking for a sound and appropriate agrotechnology to improve their income through medicinal plant cultivation in diverse conditions either monocropping or mixed cropping with agriculture etc. (Purohit 1997). However, cultivation of cereals in this region is economically not viable due to the increasing cost of human, animal power and organic manure inputs (Maithani and Nautiyal 1987). Therefore, cultivation of MAPs with mixed cropping particularly legume crops which is mostly cultivated by farmers on their marginal landscape will improve the socioeconomic status of the farmers in this region.

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